CLAIMS

What is claimed What is claimed is:

1. A method of selecting polypeptide or antibody binding moieties that are internalized into target cells, said method comprising:

i) contacting one or more of said target cells with one or more members of a phage display library;

ii) contacting members of said phage display library with a cells of a subtractive cell line;

iii) washing said target cells to remove said cells of a subtractive cell line and to remove members of said phage display library that are non-specifically bound or weakly bound to said target cells;

iv) culturing said target cells under conditions where members of said phage display library can be internalized if bound to an internalizing marker; and

v) identifying internalized members of said phage display library if members of said phage display library are internalized into one or more of said target cells.

2. The method of claim 1, wherein said phage display library is an antibody phage display library.

3. The method of claim 2, wherein said antibody phage display library displays single chain antibody Fv regions.

4. The method of claim 1, wherein said identifying comprises recovering internalized phage and repeating steps (i) through (v) to further select for internalizing binding moieties.

5. The method of claim 4, wherein said recovering comprises:

(a) lysing said target cells to release internalized phage; and

(b) infecting a bacterial host with said internalized phage to produce phage for a subsequent round of selection.

6. The method of claim 4, wherein said recovering comprises recovering nucleic acids encoding the phage-displayed antibody.

7. The method of claim 1, wherein said identifying comprises detecting expression of a reporter gene or a selectable marker.

8. The method of claim 1, wherein said cells of a subtractive cell line are present in at least 2-fold excess over said target cells.

9. The method of claim 1, wherein said target cells form an adherent layer in said method.

10. The method of claim 1, wherein step (ii) is performed at a temperature lower than step (iv).

11. The method of claim 1, wherein step (ii) is performed at about 4°C and step (iv) is performed at about 37°C.

12. The method of claim 1, wherein said phage express a selectable marker.

13. The method of claim 12, wherein said selectable marker is selected from the group consisting of a fluorescent protein, an antibiotic resistance gene, and a chromagenic gene.

14. The library of claim 13, wherein said chromagenic gene is selected from the group consisting of horse radish peroxidase, β-lactamase, luciferase, and β-galactosidase.

15. The method of claim 1, wherein said target cells are selected from the group consisting of solid tumor cells, members of a cDNA expression library, cells that overexpress a cytokine receptor, cells that overexpress a growth factor receptor, metastatic cells, cells of a transformed cell line, cells transformed with a gene or cDNA encoding a specific surface target receptor, and neoplastic cells derived from outside a solid tumor.

16. The method of claim 1, wherein said cells of a subtractive cell line are selected from the same tissue type as the target cells.

17. The method of claim 1, wherein said cells of a subtractive cell line are selected from the group consisting of fibroblasts, monocytes, stem cells, and lymphocytes.

18. A method of identifying an internalizing receptor, said method comprising:

i) contacting one or more of said target cells with one or more members of a phage display library;

ii) contacting members of said phage display library with a cells of a subtractive cell line;

iii) washing said target cells to remove said cells of a subtractive cell line and to remove members of said phage display library that are non-specifically bound or weakly bound to said target cells;

iv) culturing said cells under conditions where members of said phage display library can be internalized if bound to an internalizing marker;

v) identifying internalized members of said phage display library if members of said phage display library are internalized into one or more of said target cells;

vi) contacting the same or different target cells with the identified internalized members of step (v) or members propagated therefrom, whereby said members bind to the surface of said same or different target cells.

19. The method of claim 18 further comprising

isolating a component of the same or different target cells to which said members bind.

20. The method of claim 18, wherein said phage display library is an antibody phage display library.

21. The method of claim 20, wherein said antibody phage display library displays single chain antibody Fv regions.

22. The method of claim 18, wherein said identifying comprises recovering internalized phage and repeating steps (i) through (v) to further select for internalizing binding moieties.

23. The method of claim 22, wherein said recovering comprises:

(a) lysing said target cells to release internalized phage; and

(b) infecting a bacterial host with said internalized phage to produce phage for a subsequent round of selection.

24. The method of claim 18, wherein said cells of a subtractive cell line are present in at least 2-fold excess over said target cells.

25. The method of claim 18, wherein said target cells form an adherent layer in said method.

26. The method of claim 18,. wherein step (ii) is performed at a temperature lower than step (iv).

27. The method of claim 18, wherein step (ii) is performed at about 4°C and step (iv) is performed at about 37°C.

28. The method of claim 18, wherein said cells of a subtractive cell line are selected from the same tissue type as the target cells.

29. The method of claim 18, wherein said target cells are selected from the group consisting of solid tumor cells, members of a cDNA expression library, cells that overexpress a cytokine receptor, cells that overexpress a growth factor receptor, metastatic cells, cells of a transformed cell line, and neoplastic cells derived from outside a solid tumor.

30. A multivalent antibody phage display library, said library comprising a plurality of phage wherein said phage display, on average, at least two copies of a single-chain antibody and said library comprises a plurality of species of single-chain antibody.

31. The library of claim 30, wherein said phage display, on average, at least 4 copies of a single chain antibody.

32. The library of claim 30, wherein said library comprises at least 10^5 different species of single chain antibody.

33. The library of claim 30, wherein said antibodies are encoded by a nucleic acid that is a phage vector.

34. The library of claim 30, wherein said library is selected for members that specifically bind to an internalizing cell surface receptor.

35. The library of claim 34, wherein said cell surface receptor is selected from the group consisting of erbB2, EGF receptor, PDGF receptor, VEGF receptor, and transferrin receptor.

36. The library of claim 30, wherein said single-chain antibodies are single chain Fv or single-chain Fab antibodies.

37. The library of claim 30, wherein said phage are filamentous phage.

38. The library of claim 30, wherein said antibodies are expressed as a fusion with a PIII minor coat protein.

39. The library of claim 30, wherein said phage express a selectable marker.

40. The library of claim 30, wherein said selectable marker is selected from the group consisting of a fluorescent protein, an antibiotic resistance gene, and a chromagenic gene.

41. The library of claim 40, wherein said chromagenic gene is selected from the group consisting of horse radish peroxidase, βlactamase, luciferase, and β-galactosidase.

42. A nucleic acid library encoding an antibody library, said library comprising a plurality of phage vectors, wherein said library encodes a plurality of single-chain antibodies.

43. The claim 42, wherein said library comprises at least 10^5 different phage vectors.

44. The library of claim 42, wherein said single-chain antibodies are a single-chain Fv (scFv) or a single-chain Fab (scFab).

45. The library of claim 42, wherein said phage vectors further comprise a selectable marker.

46. The library of claim 42, wherein said library is selected for members that encode antibodies that specifically bind to an internalizing cell surface receptor.

47. The library of claim 46, wherein said receptor is selected from the group consisting of erbB2, EGF receptor, PDGF receptor, VEGF receptor, and transferrin receptor.

48. A kit comprising one or more containers containing a polyvalent phage display antibody library.

49. The kit of claim 48, wherein said phage display library contains a plurality phage vectors encoding single chain antibodies in fusion with a viral coat protein.

50. The kit of claim 48, wherein said phage display library contains a plurality of phage particles displaying, on average, at least two copies of an antibody per phage particle.

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